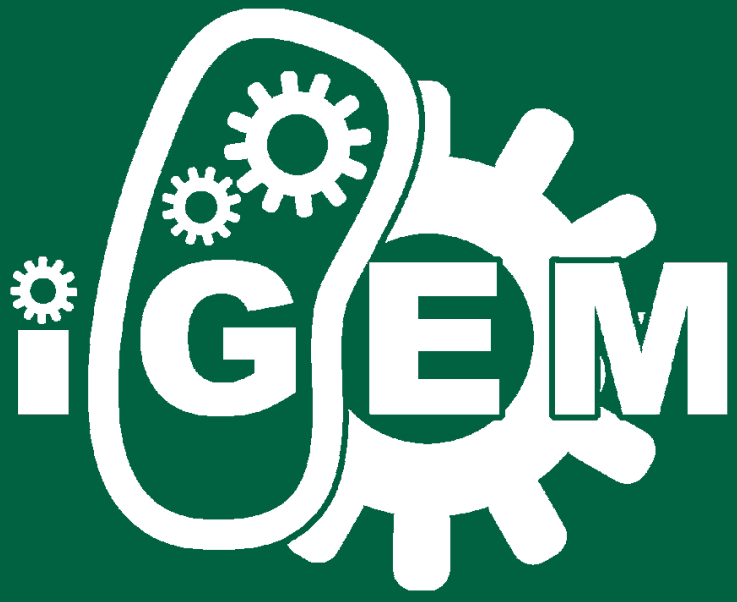
Synthetic Biology Summer Camp 2014

Wisconsin
Lutheran College



Laboratory Safety

Introduction

Lab safety is a practice that every scientist must follow. Everyone is expected to observe safe lab practices. Anyone not following safe procedure will be dismissed from lab.

Purpose

The purpose of this lab is to learn important laboratory safety procedures.

Procedure

- Safety glasses must be worn at all times in the laboratory.
- Closed-toe shoes must be worn at all times.
- Clothes must …
    - o Cover the legs down to the knees.
    - o Cover the shoulders.
    - o Cover stomach and back.
- Learn the location of all fire alarms, extinguishers, a fire blanket, eyewash stations, safety showers and the first aid kit. Also, know how to use them.
- Never eat, drink or chew gum in the lab.
- When dealing with chemical **reagents**, do not taste, sniff, or bring them near your face.
- Never sniff chemical odors directly, rather gently waft them to ward your nose.
- Avoid clutter on or around benches.
- Only perform authorized experiments.
- Nobody may work in the laboratory alone.
- Report every accident, no matter how small to your supervisor.
- Be very careful when using glassware because they are fragile.
- When reagents are spilled, tell your supervisor so the spill may be cleaned up properly.
- Do not use flammable materials near open flame.
- Be aware of what is going on around you and in the lab.
- Clean your work station carefully after each lab.

Conclusion

The purpose of this is to learn and employ important laboratory procedures. Now that you've learned the proper way of acting in a lab, you can continue to learn more techniques.

Learn to Pipette

Introduction

Pipetting is the most frequently used technique by biologists. Mastering the use of a pipette will improve experimental results, and incorrect use may end in disaster.

Purpose

The purpose of this lab is to learn how to properly use a pipette.

Further Background

P1000
•blue top
• 100-1000 µL (microliters)

P200
• gold top
• 20-200 µL (microliters)

P20
• yellow top
• 2-20 µL (microliters)

P10
• white top
• 0.5-10 µL (microliters)

P2
• white top
• 0.2-2 µL (microliters)

NOTE:
1 milliliter (ml) = 1000 microliters (µL)
Blue topped pipettes match with blue tips.
Yellow/Gold topped pipettes match with yellow tips.
White/Gray topped pipettes match with white tips.

Materials

►Water
►Pipette
►Pipette Tips
►Waste Beaker

Procedure

1. Connect correct tip to pipette.
2. Press the colored plunger of the pipette down to the first "stopping" point
3. Submerge the tip into the liquid
4. Release the top slowly to extract liquid.
5. Remove the tip from liquid
6. CAREFULLY move the pipette to where you need the liquid.

****** DO NOT TOUCH THE TIP TO ANYTHING OR YOU WILL CONTAMINATE YOUR LIQUID*****

7. To release liquid from the pipette press the colored plunger down to the first "stopping" point. (Previously done when extracting liquid).

Notice the small amount of liquid remaining in the tip of the pipette.

8. Press to the second "stopping" point to release the remaining liquid.
9. Properly dispose of your contaminated/used pipette tip into the biohazardous waste container.

Conclusion

Now you are equipped to perform various procedures that utilize micropipetting. One of these is the transformation of your plasmid into the DNA of your target organism, like we will be doing in the next lab.

Here are some extra resources on the internet to strengthen your background in pipetting.

►http://www.virtual-labs.leeds.ac.uk/pres/micropipettes/
►https://www.youtube.com/watch?v=NO1dK1zuRSw
►http://www.usc.edu/org/cosee-west/Jun07Resources/PipetteUsetraining.pdf

Transformation - Heat Shock

Introduction

We will now make use of those pipetting skills that you mastered. Transformation is the process that occurs when a cell takes in foreign DNA from its surroundings. Transformation can occur in nature within certain types of bacteria. Bacterial cells that are able to take up DNA from the environment are called competent cels.In molecular biology, transformation is artificially reproduced in the lab via the creation of pores in bacterial cell membranes. Heat shock is one method that biologists use to perform transformation in the lab.

Further Background

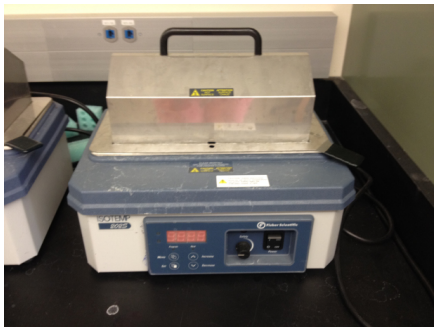
Heat shock transformation uses a calcium rich environment, provided by calcium chloride, to counteract the electrostatic repulsion between the plasmid DNA and bacterial cellular membrane. A sudden increase in temperature creates pores in the plasma membrane of the bacteria and allows for **plasmid DNA** to enter the bacterial cell. Plasmid DNA is a small, circular piece of DNA which is separate from and can replicate independently of chromosomal DNA within a cell.

Purpose

The purpose of this lab is to transform bacteria via heat shock.

Materials

- ▸Overnight grown cultures of *E. Coli Nissle 1917*
- ▸$MgCl_2$ and $CaCl_2$
- ▸Ice
- ▸Microcentrifuge Tubes
- ▸Heat Shock Apparatus
- ▸LB agar plates



Procedure

1. Obtain cultures of *E. coli Nissle* 1917 cells
2. Put the bottles of 100 mM $MgCl_2$ and 100 mM $CaCl_2$ on ice
3. Dilute the culture by adding s200 µL of culture into 5 mL fresh LB broth
4. Incubate this culture by shaking at 37° C for 1 hour.
    - #1-4 will be done for you prior to lab
5. **Pellet** the *E. coli* cells in a microcentrifuge tube.
6. **Decant** the liquid and **resuspend** the pellet in 1.5 ml in cold 100mM $MgCl_2$
7. Pellet the cells and decant
8. Resuspend the cells in 500 µL 100 mM $CaCl_2$
9. Place on ice for 20 minutes
10. Remove 100 µL of cells into a new microcentrifuge tube
11. Add 2 µL of the plasmid to 100 µL of cells
12. Place on ice for 15 minutes
13. Heat shock at 42°C for 60 seconds
14. Immediately place on ice
15. After incubating on ice approximately one minute, add 1 mL LB broth (no **antibiotic**)
16. Incubate at 37°C for 30 minutes
17. Perform a 1:10 dilution of this transformation (100 µL in 900 µL LB)

19. Plate the contents of both tubes on LB agar plates
20. Incubate these plates overnight at 37° C.
21. Plates should be removed from the incubator the following morning to examine the results.

Conclusion

Heat shock is one of many methods to transform bacteria. One other method is electroporation. It is helpful to select the method that will yield the best results for a particular experiment. Tomorrow, your bacteria will have multiplied and we can isolate the plasmid from the bacteria.

Liquid Culture

Introduction

Once you have a single **colony of bacteria**, the next step is to grow a large amount of the bacteria containing your plasmid. This is done using a liquid culture. Liquid cultures are grown using bacteria from a plate, and moving them into a broth that promotes bacterial growth. Luria broth (LB) is a nutrient-rich media commonly used to culture bacteria in the lab. LB agar plates are frequently used to isolate individual (clonal) colonies of bacteria carrying a specific plasmid. However, a liquid culture is capable of supporting a higher density of bacteria. Therefore, it is used to grow the amount of bacteria necessary to isolate enough plasmid DNA for experimental use.

Further Background

How to use an electronic pipette:

1. Acquire an electronic pipette tip (Figure 2) and open the end that will be placed into the electronic pipette while keeping the rest of the tip in the sterile packaging. DO NOT TOUCH THE END OF THE TIP TO ANYTHING EXCEPT THE SOLUTION.
    a. If the tip becomes contaminated replace it with a fresh tip.
2. Press and secure the tip into the electronic pipette.
    a. Set the speed setting on the pipette to LOW.
3. When the solution is open, remove the remaining covering on the tip and place it into the solution.
4. Press the button correlating with the up facing arrow and pipette up the desired

Figure 1: Electronic Pipette

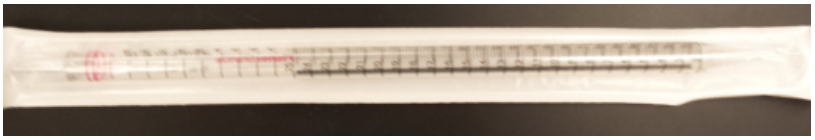


Figure 2: Electronic Pipette Tip

Purpose

The purpose of this lab is to create a liquid culture that we can then use for our next step, DNA isolation.

Materials

- ►Inoculation Loop
- ►70% EtOH
- ►Bacterial colonies (on a plate)
- ►50 ml conical tubes
- ►Sterile LB Broth
- ►Electronic Pipette
- ►25mL Electronic Pipette Tip

Procedure

1. Label the 50 mL conical tube.
2. Using an electronic pipette, pipette 30mL of sterile LB Broth into a 50mL conical tube.
3. Isolate a bacterial colony on the plate.
4. Gently press the end of a sterile wooden stick to the bacterial colony on the plate. Do not press too hard, or the agar will break.
5. Place the end of the wooden stick into the LB Broth and spin the stick for 15 seconds.
6. Securely cap the conical tube and place on a shaker in a warm room overnight.
    a. NOTE: A shaker is used to promote bacterial growth due to the constant agitation.

Figure 3: Shaker

Conclusion

Now comes waiting. Tomorrow, your conical tube should appear cloudy in nature- this means more bacteria! We will then use this to advance further down the road of synthetic biology – isolating our new plasmid. This will be done by performing a "mini prep."

Plasmid Purification: Mini Prep

Introduction

For synthetic biology, **plasmid purification** is important because it allows for the isolation of DNA from cells, which can then be analyzed and used for other lab protocols. For example, the iGEM team used this protocol to separate DNA from bacteria. They then combined it with other DNA to create a longer strand, called a part or a gene.

Further Background

There are several ways to purify plasmids. These methods are often called "mini preps" because you are purifying DNA from a small volume of cells. Mini preps involve lysing, or breaking, the cells and purifying the DNA via purification and/or membrane binding. The method we will do uses a silica-gel membrane to bind the DNA. You will purify the plasmid DNA and analyze it.

This protocol is designed for purification of up to 20 µg of **high-copy plasmid** DNA from overnight cultures (1-5 ml) of *E. coli* in LB medium. This protocol can be used for the purification of **low-copy plasmids** and **cosmids**, large plasmids (>10 kb), and prepared DNA.

Purpose

In this lab you will purify the plasmid DNA of *Escherichia coli* and analyze it.

Materials

- ►Plasmid infected *E. Coli*
- ►7X Lysis **Buffer** (Blue)
- ►Neutralization Buffer (Yellow)
- ►Endo-Wash Buffer
- ►Zyppy Wash Buffer
- ►Zyppy Elution Buffer
- ►Zymo-Spin IIN Columns
- ►Collection Tubes
- ►QIAprep spin columns
- ►Microcentrifuge Tubes

Procedure

1. Note: All protocol steps should be carried out at room temperature.
1. Pellet the bacterial culture.
2. Decant (pour out) the broth from the 15mL conical tube, leaving the pellet in the bottom of the tube.
3. Resuspend (pipette up and down) the pellet by adding ~525µL DI water to the same tube. After it has been resuspended, use a pipette to transfer the contents to a microcentrifuge tube (it should end up near 600µL total). Label the tube with your initials.
   i. When resuspending, do not pipette too vigorously so bubbles do not form.
4. Add 100µL 7x Lysis Buffer to the microcentrifuge tube and mix thoroughly by inverting the tube 4-6 times.

Available: http://public.callutheran.edu/~revie/protocols/Q-Minipreps.pdf. Accessed 20 October 2013.

i. Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

ii. After addition of the 7X Lysis Buffer the solution should change from opaque to clear blue, indicating complete lysis.

5. Immediately add 350 µL of Neutralization Buffer to the microcentrifuge tube and mix thoroughly by inverting the tube 4–6 times.

i. To avoid localized precipitation of the DNA, mix the solution thoroughly, immediately after addition of the Neutralization Buffer. Large culture volumes (e.g. ≥5 ml) may require inverting up to 10 times. The solution should become cloudy.

ii. The sample will turn yellow when the neutralization is complete and a yelowish precipitate will form.

6. Centrifuge for 3 min at 13,000 rpm in a table-top microcentrifuge.

7. A compact white pellet will form.

8. Apply the supernatants from step '6' to the Zymo-Spin IIN column by decanting (pouring).

i. The supernatant is the fluid surrounding the pellet.

9. Place the column into a Collection Tube and centrifuge for 30 seconds. Discard the flow-through (the liquid which accumulates at the bottom of the column).

10. Wash the QIAprep spin column by adding 200µL of Endo-Wash Buffer to the column. Centrifuge for 30 seconds.

11. Add 400µL of Zyppy Wash Buffer to the column. Centrifuge for 1 minute.

12. Transfer the column into a clean 1.5mL microcentrifuge tube then add 30µL of Zyppy Elution Buffer directly to the column matrix and let stand for one minute at room temperature.

13. Centrifuge for 30 seconds to elute the plasmid DNA.

14. Label and place your tube in the -20°C freezer for future use or immediately characterize the product using Gel Electrophoresis.

Conclusion

In this lab you learned how to isolate DNA from bacteria. In order to further use this product, you need to know the concentration of DNA that is present in your tube.

DNA Quantification: Qubit® dsDNA Assay Kit

Introduction

Quantification of DNA is extremely useful in the microbiology lab. It is used to find the quantity and concentration of DNA. Our iGEM team uses this protocol to find the concentration of DNA in our samples.

Purpose

In this lab you will determine the amount and concentration of isolated plasmid. We will use this concentration for future experiments.

Materials

- ►Qubit® dsDNA BR Reagent
- ►Qubit® dsDNA BR Buffer
- ► Qubit® dsDNA BR Standard #1
- ► Qubit® dsDNA BR Standard #2
- ► Qubit Tubes
- ► Qubit Fluorometer
- ►QIAprep spin columns
- ►Microcentrifuge Tubes

Procedure

1. Note: make sure all mixtures are at room temperature before using the Qubit machine.
2. Make the working solution by mixing 1 part Qubit dsDNA BR Reagent with 199 parts Qubit dsDNA BR Buffer in a conical tube.
    a. Make enough working solution for all of your samples plus one extra sample.
3. Label a Qubit tube Standard #1. Make standard 1 by adding 190 µL working solution to the tube. Add 10 µL Qubit dsDNA BR Standard #1 to the tube. Vortex solution.
4. Label a Qubit tube Solution #2. Make solution 2 by adding 190 µL of working solution to the tube. Add 10 µL Qubit dsDNA BR Standard #2 to the tube. Vortex solution.
5. Label a Qubit tube Sample A. Add 199 µL working solution to the tube. Add 1 µL of an unknown DNA sample to the tube.
6. Repeat step "5" for each of your DNA samples, labeling them in sequence (e.g. Sample B, Sample C, etc.)
7. Measure fluorescence using the Qubit Fluorometer.
    a. Hit any button on the fluorometer to turn it on.
    b. Use the arrows to highlight Quant-iT dsDNA, BR. Hit GO.
    c. Follow the on-screen directions, which include the insertion of Solution #1 and Solution #2. These are done to calibrate the fluorometer to your samples.
    d. Add Sample A to the fluorometer. Hit GO. Record displayed concentration of DNA.
    e. Repeat step "d" for your remaining DNA samples.

Conclusion

In this lab you used a Qubit dsDNA assay kit to determine what concentration of DNA was in your samples. From here, you can crunch some numbers to determine how much of this sample to use when combining it with a restriction enzyme in order to cut the DNA. This is called a restriction digest.

Working Solution Calculations

(# of samples) x (199) = ____ µL Qubit dsDNA BR Buffer

(# of samples) x (1)= _____ µL Qubit dsDNA BR Reagent

When you are doing multiple samples, a working solution cuts down on the amount of steps to carry out quantification.

PCR: Polymerase Chain Reaction

Introduction

A Polymerase Chain Reaction is a way to generate exponential copies of a selected piece of a DNA molecule. This is important because it enables you to make copies of your selected molecule efficiently.

Further Background

a. The objective of PCR is to produce a relatively large amount of a specific piece of DNA from a small amount of **template DNA**. A researcher can use PCR to amplify trace amounts of DNA from a drop of blood or a single hair follicle to generate millions of copies of a desired DNA fragment.

b. PCR makes use of two basic processes in molecular genetics:

i. Complementary DNA strand hybridization

ii. DNA strand synthesis via **DNA polymerase**

c. Before a region of DNA can be amplified, one must identify and determine the sequence of a piece of DNA **upstream and downstream** of the region of interest. These areas are then used to make the **oligonucleotide primers** that will serve as starting points for DNA replication. Primers are required to provide a double-stranded starting point for the DNA polymerase.

d. The DNA polymerase used in PCR, however, must be a thermally stable polymerase because the polymerase chain reaction cycles between temperatures of ~60°C and ~94°C. A thermo-stable DNA polymerase (Taq polymerase) from the **thermophilic** bacteria, *Thermus aquaticus*, is commonly used for this purpose.

e. Following sample preparation, the template DNA, oligonucleotide primers, thermo-stable DNA polymerase, the four deoxynucleotides (A, T, G, C), and reaction buffer are mixed in a single PCR tube. The tube is placed into the thermal cycler. Thermal cyclers contain an aluminum block that holds the samples and can be rapidly heated and cooled across extreme temperature differences.

Temperature Cycle

1. **Denaturation**
   -strands separate from high temperature (94°C)
2. **Annealing**
   -primers attach to the separated DNA (50-60°C)
3. **Extension**
   -DNA polymerase extends primers to copy the DNA (72°C)
4. Repeat ~ 40 times

f. Thermal cycling continues for 40 cycles. After each thermal cycle the number of template strands doubles, resulting in an exponential increase in the number of template DNA strands. After 40 cycles there will be approximately 1.1×10^{12} more copies of the original number of template DNA molecules.

Purpose

The goal of this lab is to design and execute a PCR reaction to amplify Tse2 toxin from the plasmid BBa_075001.

Materials

►PCR Tubes
►PCR Tube Rack
►Master Mix
    a. DMSO
    b. DNA polymerase
►10uM Forward Primer
►10uM Reverse Primer
►Template DNA
►Thermal Cycler

Procedure

1. Carefully label a 0.2 ml PCR tube.
    a. NOTE: Handle the PCR tube with care as it cracks easily.
    b. NOTE: Keep the master mix in the cryobox at all times. Keep the cryobox in the freezer as much as possible.
2. Add 12.5 µL master mix to the PCR tube.
3. Add 2.0 µL of the DNA Template to the PCR tube.
4. Add 8 µL DepC Water to the PCRtube.
5. Add 1.25 µL Primer A (TBD) and 1.25 µL Primer B (TBD) to the PCR tube.
6. Gently pipette up and down to mix the solution.
7. After mixing, place your microfuge tube in the thermal cycler. The full process (~98 min), which is performed, is outlined below.
    a. Hot Start, 98°C, 3 min
    b. Denaturation, 98°C, 30sec
    c. Annealing, 67°C, 30sec
    d. Extension, 72°C, 2min
    e. Repeat 2-4 40 times
    f. Final Extension, 72°C, 5min
    g. Hold, 4°C, Infinity
8. Store your PCR product at -20°C for **characterization** and cloning during the lab.

Conclusion

The purpose of this lab was to design and execute a PCR reaction to amplify the Tse2 toxin from the plasmid BBa_875001. This is important because it allows us to produce copies of the selected molecule.

Restriction Enzyme Digest

Introduction

Restriction digestion is an important process in the microbiology lab because it allows DNA to be cut in a specific spot. The restriction enzymes used will cut the DNA at positions where a certain sequence of base pairs is present. The iGEM team used this protocol to **cleave** their DNA samples. This prepared the samples to be combined with other DNA samples.

Purpose

In this lab you will use restriction digestion to cleave two DNA samples.

Materials

- ►Ice and bucket/container
- ►PCR Tubes
- ►Part A (Purified DNA, > 16ng/ul)
- ►Part B (Purified DNA, > 16ng/ul)
- ►dH2O
- ►NEBuffer 2.1 (10x)
- ►Restriction Enzymes: EcoRI, SpeI, XbaI, PstI
- ►**Thermal cycler**

Procedure

1. Put the Buffers used on ice. Keep the enzymes in the -20°C freezer until needed. When using the enzymes, place them in the ice and ensure the bottom of the enzyme tubes are below the ice.
2. Thaw NEBuffer 2.1. Mix by pipetting up and down. Centrifuge as needed.
3. Label one PCR tube 'Part A' and another 'Part B'. Add the specified amount DNA Part A to the tube 'Part A' and the specified amount of DNA Part B to the tube 'Part B'. Add sterile water to the tubes for a total volume of 16.5µL in each tube.
    a. Calculation example (with 25ng/µL as DNA sample concentration): 250ng ÷ 25ng/µL = 10µL of DNA sample. 16.5µL (total volume) – 10µL (DNA sample) = 6.5µL of sterile water
4. Pipette 2.0µL of NEB Buffer 2.1 to each tube.
5. In the Part A tube: Add 0.5uL of EcoR, and 0.5µL of Spe.
6. In the Part B tube: Add 0.5uL of Xba, and 0.5µL of Pst.
7. The total volume in each tube should be approximately 20µL. Mix well by pipetting slowly up and down. Spin the samples briefly to collect all of the mixture to the bottom of the tube.
8. Incubate the restriction digests at 37°C for 30 minutes, then 65°C for 20 minutes. We use a thermal cycler with a heated lid.
    a. RUN ON THERMAL CYCLER PROTOCOL IGEMP002
9. Keep sample at 4°C if not to be used immediately or in the -80°C for long term storage.

	Part A	*Part B*
DNA	*500ng: ____ µL*	*500ng: ___ µL*
dH2O	*adjust to 20 µL*	*adjust to 20 µL*
NEB Buffer 2.1	*2.0 µL*	*2.0 µL*
Enzyme 1	*0.5 µL EcoR1*	*0.5 µL XBa1*

Conclusion

In this lab you used restriction enzyme digestion to cleave DNA. These cut DNA parts are now able to be combined with other DNA.

Gel Electrophoresis

Introduction

Gel electrophoresis is a widely used technique for separating and analyzing nucleic acids. This technique is a broadly applied research tool; for example, it can be used clinically, forensically, or in biotechnology.

In previous years, the iGEM team has used electrophoresis often to confirm the size of specific strands of DNA. Agarose gel electrophoresis was used to verify the size and identy of DNA to start the project as well as a check to confirm that specific DNA manipulation steps (for example: Miniprep, PCR, Digest...) were successfully performed.

Further Background

During gel electrophoresis, an electric current is applied. The negatively charged nucleic acids are pulled through a gelatinous matrix toward the positive cathode. Since the gel is slightly porous, smaller nucleic acids are able to migrate through the gel faster than larger nucleic acids, thereby separating the nucleic acids by size.

You wil be using the DNA samples that were digested yesterday during the Restriction Digestion. We are able to tell the base pair length by using a standardized group of DNA (the ladder) which we will use to find the base pair length of the Restriction Digestion product.

Purpose

The goal of this lab is to use DNA electrophoresis to separate and analyze DNA from multiple samples.

Materials

- ►Agarose
- ►Electrophoresis chamber and power supply
- ►Gel mold
- ►Gel comb
- ►DNA loading dye
- ►1x TAE buffer
- ►GelGreen Nucleic acid stain
- ►DNA samples
- ►DNA 1 kb ladder (NEB)
- ►Microwave
- ►250ml Erlenmeyer Flask
- ►Kimwipe

Procedure

Prepare Agarose Gel

1. NOTE: Gels have been premade in the interest of time; however, this protocol outlines the procedure for preparing agarose gels for students interested in the procedure.
2. Prepare a 0.8% agarose gel by heating 0.4g agarose in 50mL 1xTAE in the microwave (~30sec to 1min on full power) until completely dissolved. Use caution to not burn yourself or boil the solution over (an Erlenmeyer flask with a Kimwipe stuffed in the neck will help to prevent this). Be sure that there are no "chunks" of agarose remaining that have not yet dissolved, as this will distort the migration of the nucleic acids through the gel.
3. Once the gel has cooled so that it is comfortable to touch the flask with your gloved hand, add GelRed or GelGreen DNA stain to a 2x concentration (1 µL per 50mL gel).
4. CAUTION: GelGreen and GelRed DNA stains are suspected carcinogens. Handle with caution and be sure to wear gloves!
5. Place the gel mold into the caster and tighten the rubber gasket. Pour the dissolved agarose into the gel mold and place comb in one end. (Be careful not to wait too long or the gel will start to solidify in the flask.) Pour slowly to avoid air bubbles or use the comb to push bubbles to the side of the mold. Allow the gel to solidify (this will typically take about 5-10 minutes).
6. Once the gel has solidified (a cloudy appearance is a good indication, but check with a gloved finger), remove the comb. Place the gel in the electrophoresis rig with the wells closest to the negative (black) end and cover with 1x TAE buffer. Use a minimal amount of buffer to cover the gel to maximize the rate of electrophoresis and conserve TAE (this requires ~250mL of 1x TAE buffer).

Gel Electrophoresis

1. Check that the wells of the agarose gels are near the black (-) electrode. The negatively charged DNA and tracking dyes will migrate toward the red (+) electrode. (RUN TOWARD RED is a helpful phrase to remember this.)

2. Add 1 µL of the loading dye to each of the sample DNAs (~5 µL). Add 1µL of loading dye to the "Ladder" DNA in the first lane of the gel, add this combined 2 µL to the first lane. In the second lane, load the sample 1 DNA. In the 3rd, 4th and 5th lanes load the DNA for sample 2, 3, and 4, respectively.

3. Turn on the power supply, and run the gel at 100V until the dye is about 75% of the way through the gel (~50min).

4. Remove gel from the well, and place on a UV light box.

5. Take a picture of the DNA samples using a camera; please use only clean ungloved hands when touching the camera! Put your gloves back on to handle the gel.

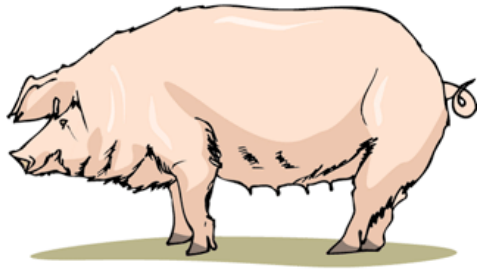
Conclusion

In this lab you learned to use DNA electrophoresis to separate and analyze DNA from multiple samples. This allows you to accurately identify specific DNA molecules so you can continue your project knowing that you are using the correct components.

Fetal Pig Dissection

Introduction

Now that you have learned how to genetically engineer organisms, it is important to understand how that can affect nature. For example, the past year's iGEM Team had a target organism for their probiotic: the goat. They needed to understand anatomic structure and the chemical nature of the goat's stomach. The chief way that people learn about anatomy is by dissection. Today, we will be dissecting a fetal pig to bring our lab sessions together to a close.



Purpose

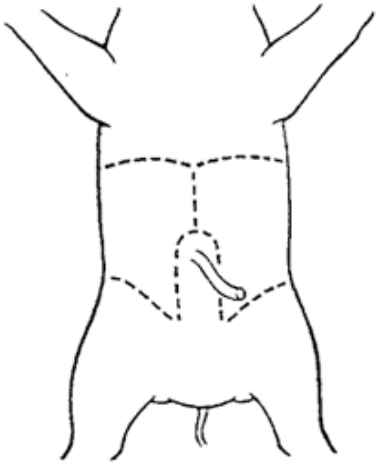
The purpose of this lab is to explore the anatomy of a fetal pig through dissection.

External Anatomy

1. Determine the sex of your pig by looking for the urogenital opening. On females, this opening is located near the anus. On males, the opening is located near the umbilical cord.
    a. If your pig is female, you should also note that urogenital papilla is present near the genital opening. Males do not have urogenital papilla.
    b. Both males and females have rows of nipples, and the umbilical cord will be present in both.
    c. What sex is your pig? _____
2. Make sure you are familiar with terms of reference: **anterior, posterior, dorsal, ventral**. In addition, you'll need to know the following terms
    a. **Medial:** toward the midline or middle of the body
    b. **Lateral:** toward the outside of the body
    c. **Proximal:** close to a point of reference
    d. **Distal:** farther from a point of reference

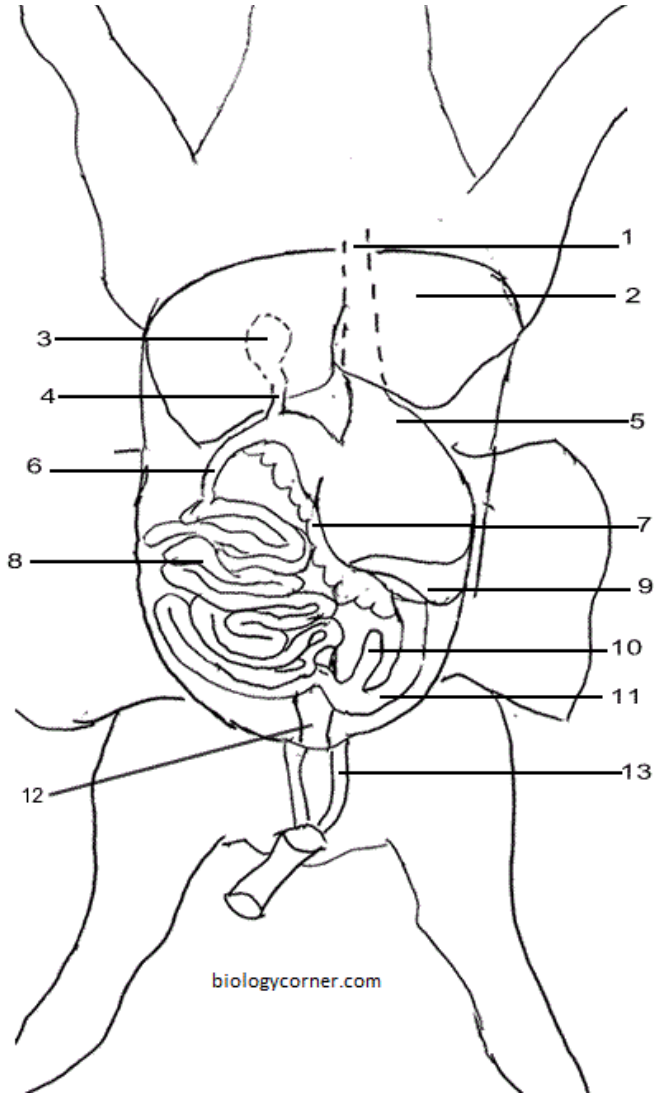
First Incision

1. Place your fetal pig in the dissecting pan ventral side up. Use string to tie your pig so that the legs are spread and not in your way. Use scissors to cut through the skin and muscles according to the diagram. Do not remove the umbilical cord. In the first section, you will only examine the abdominal cavity (the area below the ribcage).
2. After completing the cuts, locate the umbilical vein that leads from the umbilical cord to the liver. You will need to cut this vein in order to open up the abdominal cavity.
3. Your pig may be filled with water and preservative, drain over the sink if necessary.Locate each of the organs on the next page.



Abdominal Cavity

1. Diaphragm.This muscle divides the thoracic and abdominal cavity and is located near the rib cage. The diaphragm aids in breathing.
2. *Liver.* This structure is lobed and is the largest organ in the body. The liver is responsible for making bile for digestion.
3. *Gall bladder.* This greenish organ is located underneath the liver; the bile duct attaches the gall bladder to the duodenum. The gall bladder stores bile and sends it to the duodenum, via the bile duct.
4. *Stomach*. A pouch shaped organ that rests anitomical left. At the top of the stomach, you'll find the esophagus. The stomach is responsible for churching and breaking down food.
5. View the inside of the stomach by slicing it open lengthwise.
6. The stomach leads to the *small intestine*, which is composed of the duodenum (straight portion just after the stomach) and the ileum (curly part), and the jujuneum.
7. The ileum is held together by mesentery. In the small intestine, further digestion occurs and nutrients are absorbed through the arteries in the mesentery. These arteries are called mesenteric arteries.
8. *Pancreas*: a bumpy organ located along the underside of the stomach, a pancreatic duct leads to the duodenum. The pancreas makes insulin, which is necessary for the proper uptake of sugars from the blood. The pancreas also produces enzymes which break down the proteins, lipids, carbohydrates, and nucleic acids in food.
9. *Spleen*: a flattened organ that lies across the stomach and toward the extreme anatomical left. The spleen stores blood and is not part of the digestive system. On the underside of the spleen, locate the splenic artery.
10. At the end of the jujuneum, where it widens to become the large intestine, a "dead-end" branch is visible. This is the *cecum*. The cecum helps the pig digest plant material.
11. The *large intestine* can be traced to the rectum. The rectum lies toward the back of the pig and will not be moveable. The rectum leads to the anus, which opens to the outside of the pig. The large intestine reabsorbs water from the digested food, any undigested food is stored in the rectum as feces.
12. Lying on either side of the spine are two bean shaped organs: the *kidneys*. The kidneys are responsible for removing harmful substances from the blood, these substances are excreted as urine. (more on this later)
13. Two *umbilical vessels* can be seen in the umbilical cord, and the flattened urinary bladder lies between them.

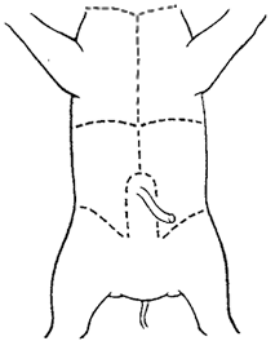


Identify the structures on the diagram.

1._____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____
9. _____
10._____
11._____
12. _____
13._____

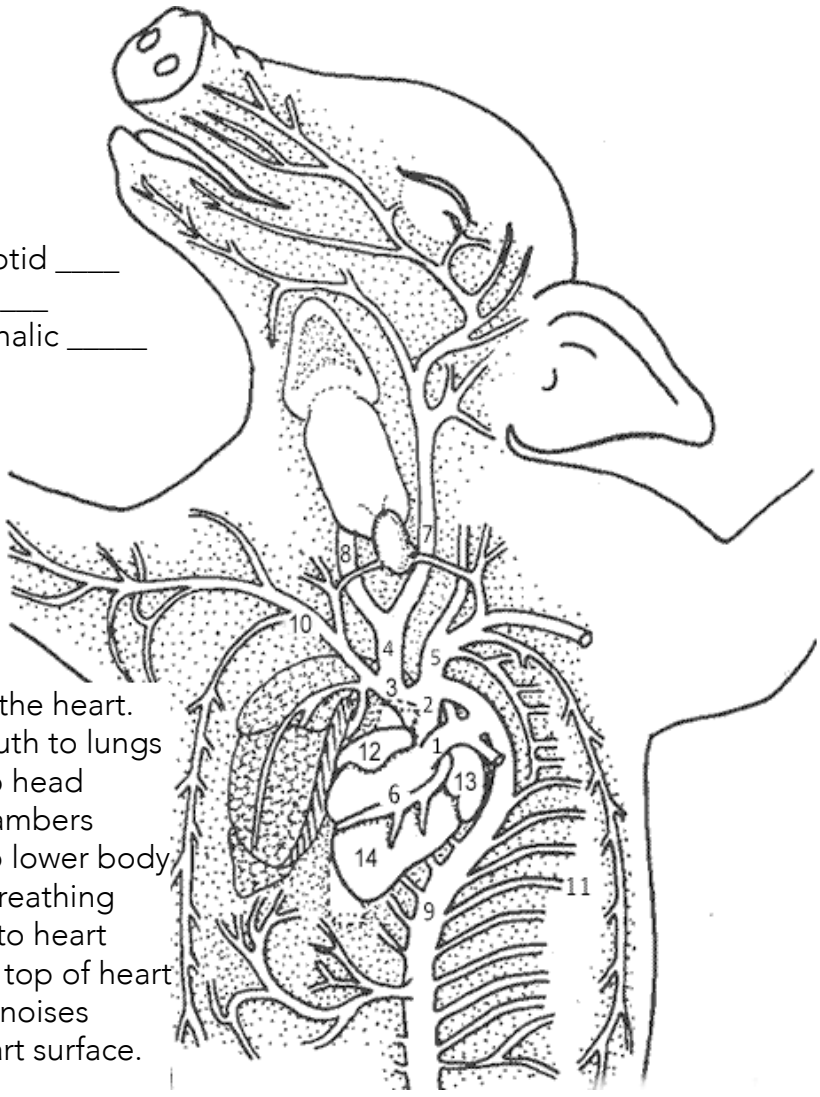
Thoracic Cavity

1. Find the diaphragm again. Remember that the diaphragm separates the abdominal cavity from the thoracic cavity and it aids in breathing. Above the diaphragm is the heart.
2. Remove the pericardium, which is a thin membrane that surrounds the heart.
3. The structures visible on the heart are the two atria (12,13), the ventricle (14) which has two chambers not visible from the outside.
4. The most obvious vessel on the top of the heart is the pulmonary trunk (1), it curves upward and joins the aorta (2) - a vessel which arches from the heart and curves around to go to the lower part of the body -where it is called the abdominal (dorsal) aorta (9). The aorta supplies the body with blood.
5. The aorta will curve back and then branch in two spots – the right brachiocephalic (3) and the left subclavian (5)
6. The right brachiocephalic then branches into arteries – the common carotid (4) and the right subclavian (10) The sublavians supply blood to the arms and follow the clavicle bone
7. The common carotid will branch into the left (7) and right carotid arteries (8). The carotid arteries supply blood to the head and neck.



8. Observe the coronary vessels (6) on the outside of the heart - these vessels supply blood to the muscle of the heart.
9. Easy arteries to find are the ones that run near the ribs. These are the intercostal arteries (11).
10. Lift the heart to look on its dorsal side (toward the back), you should be able to see the anterior and posterior vena cava, which brings blood from the body back to the heart. In addition, you should also be able to find the left and right jugular veins that drain blood from the head and run parallel to the carotids.
11. Push the heart to the side to locate two spongy lungs. The lungs are connected to bronchial tubes (not visible) which connect to the trachea (forming an up side-down Y).
12. The trachea is easy to identify due to the cartilaginous rings, which help keep it from collapsing as the animal inhales and exhales. The trachea should be locat ed in the neck.
13. Lying atop the trachea, locate the pinkish-brown, V shaped structure called the thyroid gland. This gland secretes hormones that control growth and metabolism.
14. At the anterior (toward head) of the trachea, you can find the hard light colored larynx (or voice box). The larynx allows the pig to produce sounds - grunts and oinks.

Aorta ____ Dorsal Aorta ____
Common carotid ____ Left & Right Carotid ____
Coronary vessels ____ Left Subclavian_____
Right Subclavian _____ Right Brachiocephalic _____
Right Atrium _____ Left Atrium _____
Intercostal _____ Ventricle ____



Identify the structure.

1. _____ Membrane over the heart.
2. _____ Airway from mouth to lungs
3. _____ Blood supply to head
4. _____ Lower heart chambers
5. _____Blood supply to lower body
6. _____Muscle to aid breathing
7. _____ Returns blood to heart
8. _____ Large vessel at top of heart
9. _____ Used to make noises
10. _____ Arteries on heart surface.

Glossary

Lab Safety

Reagent: A substance or mixture used for chemical reactions.

Transformation - Heat Shock

Plasmid DNA: DNA that is contained within a plasmid.
Pellet: Using a centrifuge to compress solid material into a small pellet at the bottom of the test tube.
Decant: To pour out the liquid within a test tube.
Resuspend: To place cells or particles in suspension in a fluid again.
Antibiotic: An antibiotic used in the laboratory is meant to select for bacteria which have a particular antibiotic resistance and to put pressure on bacteria to keep them from ejecting an inserted plasmid.

Liquid Culture

Bacterial Colony: A visible cluster of bacteria growing on the surface of a medium. The entire colony presumably grew from a single bacterial cell.

Mini Prep

Plasmid Purification: The isolation of a plasmid from a bacterial cell.
High Copy Plasmid: A plasmid which is replicated quickly within a cell.
Low Copy Plasmid: A plasmid which is replicated slowly within a cell.
Cosmid: A type of plasmid into which large strands of DNA can be inserted.
Buffer: A solution used to prevent changes in pH. A change in pH could lead to molecule destruction.

PCR

Template DNA: The original DNA molecule which will be copied via PCR.
DNA polymerase: An enzyme that creates DNA molecules by assembling nucleotides.
Nucleotide: The building block of DNA.
Upstream or Downstream: A relative position in DNA or RNA. Each strand of DNA or RNA has both a 3' and a 5' end. Relative to the position on the strand, downstream is the region towards the 3' end of the strand and upstream is the region towards the 5' end of the strand. Since DNA strands run in opposite directions, downstream on one strand is upstream on the other strand.
Oligonucleotide primer: A single stranded piece of DNA which is complementary to the original DNA strand and is used in PCR as a start point for replication.
Thermophilic: A thermophilic organism is an organism that thrives at extremely high temperatures.
Denaturation: The step in which the weak Hydrogen bonds between the two strands of DNA are broken, yielding single-stranded DNA molecules.
Annealing: When the DNA polymerase binds to the primer template and begins DNA formation.
Extension: The step in which the DNA polymerase synthesizes an new DNA strand complementary to the template strand.
Characterization: In this circumstance, using gel electrophoresis to confirm the base pair length of your PCR product.

Restriction Digest

Cleave: To sever a piece of DNA at a specific sequence of nucleotides.
Thermal Cycler: A laboratory apparatus most commonly used to amplify DNA segments via PCR.

Gel Electrophoresis

Cathode: The positively charged electrode to which electrons (negatively charged) flow.

Pig Dissection

Anatomical Position: The standing position in which the arms are at the side with the palms facing forward. All naming is based on positions relative to the body in the Anatomical Position.
Anterior: Toward the front.
Posterior: Toward the back.
Dorsal: Directed towards the back/ spinal column.
Ventral: Directed towards the belly.
Medial: Toward the midline or middle of the body.
Lateral: Toward the outside of the body.
Proximal: Close to a point of reference.
Distal: Farther from a point of reference.